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А





WT









KO

D



Е

Rotarod Test



Grip Strength Test



Appendix Figure S1. Models of three-chamber social interaction test and Elevated-plus maze test and tests of motor ability.

- (A)The model of the social interaction test.
- (B)Representative motion trail from 'Stranger 1-Empty' and 'Stranger 1- Stranger 2'.
- (C)Representative motion trail from the Elevated-plus maze test.
- (D) The rotarod test shows that the forced locomotor activity is not significantly different between WT and KO mice. n=8 mice, independent replicates.
- (E) The grip strength is no significant difference between WT and KO mice. n=8 mice, independent replicates.

Date information: Error bars represent means \pm S.E.M.; Two-tailed unpaired t-test. n.s., not significant.

Appendix Figure S2



Appendix Figure S2. The expression of NKX2.2 in NPCs.

- (A) Immunofluorescence staining for PAX6 and TBR2 during the E13 period in KO and WT. Scale bar represents 50µm.
- (B) Statistics of PAX6⁺ cells per 100µm2 surface of VZ/SVZ. n=5 mice, independent replicates.
- (C) Statistics of TBR2⁺ cells per 100μm² surface of VZ/SVZ. n=5 mice, independent replicates.
- (D) Neuron progenitor cell cultured in vitro were immunostained with anti-NKX2-2 and anti-SOX2 or anti-TUJ1 antibodies, respectively. Scale bar represents 50µm.
- (E) Immunofluorescence staining for NKX2.2 in the cerebral cortex at E13, E5 and P0. Scale bar represents 100µm.
- (F) Neuron progenitor cell of WT and KO cultured in vitro were immunostained with anti-NKX2.2 and anti-mH2A1.2. Scale bar represents 50µm.
- (G) Immunostaining shows that the expression of Nkx2.2 is decreased in mH2A1.2 brains at E13. Scale bar represents 50μm.

Date information: Representative images from at least three independent experiments.

Error bars represent means \pm S.E.M.; Two-tailed unpaired t-test. n.s., not significant.

Appendix Figure S3



Appendix Figure S3. The knockdown of NKX2.2 increases the proliferation of neural progenitor cell.

- (A) The cluster of two WT and KO.
- (B) Enrichment analysis of the biological processes based on the RNA-seq results that identified the upregulated genes.
- (C) Enrichment analysis of the biological processes based on the RNA-seq results that identified the downregulated genes.RNA-seq results that identify the downregulated and upregulated genes. The log(P-values) are indicated by the bar plots.
- (D) The volcano map show the gene profiling expression. Gene analysis revealed that the transcript levels of genes were upregulated or downregulated by more than twofold between the KO and the WT cells. n= 2 biological replicates.
- (E) Expression of NKX2.2 at the WT and KO mRNA levels was analysed by qRT-PCR.n = 6 mice, independent replicates.
- (F) Western blot analysis shows that exogenous Flag-NKX2-2 is efficiently reduced in NKX2-2-shRNA transfected 293FT cells.
- (G) Graph shows that the amount of NKX2.2 is obviously decreased in NKX2.2shRNA transfected 293 cells. n = 5 samples, independent replicates.
- (H) Brain sections of Control and NKX2.2-shRNA mice at E16.5 were immunostained with mitotic marker pH3 and DAPI. Scale bar represents 20µm.
- Statistics of pH3⁺ GFP⁺ double positive cells in VZ/SVZ. n=8 mice, independent replicates.
- (J) Representative images of E16.5 coronal brain sections were immunostained for PAX6. The control and NKX2.2-shRNA plasmid was electroporated into E13.5 mouse brains and the mice were sacrificed at E16.5. Scale bar represents 50µm.
- (K) Quantification of GFP⁺ PAX6⁺ double positive cells compare with total GFP⁺ in the VZ/SVZ. n=5 independent replicates.



D



Appendix Figure S4. mH2A1.2 binds to Brd4 to activate NKX2.2 to mediated neurogenesis.

- (A) Schematic of different regions (from-5000bp to CDS) of the NKX2.2 locus selected for ChIP-qPCR
- (B) mH2A1.2 promotes Brd4-mediated H3K27ac modification. The HA-mH2A1.2 plasmid was co-transfected with Flag- Brd4 or control into N2A cells. The cell lysates were immunoprecipitated with anti-HA, and bound proteins were detected by western blotting with the indicated antibodies.
- (C) The HA-mH2A1.2 plasmid was cotransfected with Flag-Brd4 or control into N2A cells. The cell lysates were immunoprecipitated with anti-Flag, and bound proteins were detected by western blotting with the indicated antibodies.
- (D)The model for mH2A1.2 mediated neurogenesis. mH2A1.2 aggregation and Brd4 start H3K27 acetylation to increases the transcriptional expression of NKX2.2, mH2A1.2 deficiency causes abnormal neurogenesis and dendritic morphology of neurons, and autism-like behavior in mice.